

GC-MS Metabolite Profiling, Antibacterial, Antidiabetic and Antioxidant Activities of Brown Seaweeds, *Sargassum wightii* Greville Ex J. Agardh, 1848 and *Stoechospermum marginatum* (C. Agardh) Kützing 1843

Paramasivam Deepak¹, Muthu Paulraj Diviya Josebin², Rajasekaran Kasthuridevi¹, Rajamani Sowmiya³, Govindasamy Balasubramani¹, Dilipkumar Aiswarya¹, Pachiappan Perumal^{1*}

¹Department of Biotechnology, School of Biosciences, Periyar University, Salem-636 011, Tamil Nadu, INDIA.

²Department of Microbial Biotechnology, School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbatore-641046, Tamil Nadu, INDIA.

³Greensmed Labs, Thoraipakkam, Chennai-600 097, Tamil Nadu, INDIA.

ABSTRACT

Objectives: The present study focuses on antibacterial, antidiabetic and antioxidant activities of methanolic extracts of brown seaweed *Stoechospermum marginatum* (SMME) and *Sargassum wightii* (SWME). **Methods:** The antibacterial activities of the seaweed extracts were determined by agar well diffusion method. Phytochemical, antioxidant and antidiabetic activity of the selected seaweed extracts were performed. **Results:** The phytochemical analysis of SMME and SWME has confirmed the presence of fixed oil, fat, tannin, flavonoids, alkaloids, steroids, phenol compounds, saponin etc. The SWME showed maximum activity against *Staphylococcus aureus* and minimum activity against *Escherichia coli*. And the SMME has shown moderate activity against *S. aureus*. The antidiabetic efficacy of SWME revealed the maximum effect with the inhibitory concentration value (IC₅₀: 58.36 µg/mL) followed by SMME. The SWME has showed the highest scavenging property in all the tested assays (DPPH, FRAP and H₂O₂) in relation to the control, ascorbic acid. The bioactive metabolites of the extracts were chemically characterized by FTIR and GCMS analy-

ses. GC-MS analysis of SWME revealed the presence of a major chemical compound, hexadecenoic acid, methyl ester (13.35%) which might be responsible for the recorded activity. The FTIR spectrum analyses of crude extracts revealed the presence of alkyl halides, alkanes, amides, aromatics and carboxylic acids. Hence, the present study could form a base-line for the effective biomedical utilization of the seaweed, *S. wightii*.

Key words: Antidiabetic, Antioxidant, Brown seaweeds, *Sargassum wightii*, *Stoechospermum marginatum*.

Correspondence:

Prof Pachiappan Perumal,

Department of Biotechnology, School of Biosciences, Periyar University, Salem-636 011, INDIA.

Phone no: +91 9443986669

Email: perumalarticles@gmail.com

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INTRODUCTION

Marine environment is an excellent source of reservoir for biologically active natural products, which exhibits structural features that have not been found in terrestrial natural products.¹ The secondary metabolites of marine organisms have been used for variety of purposes like food, fragrance, insecticides, medicines and pigments.² Marine organisms contain highly bioactive secondary metabolites that might represent useful leads towards the development of new pharmaceutical agents. In recent years, many bioactive compounds have been extracted from various marine plants, animals and microbes. Approximately 2500 new metabolites have been reported from a variety of marine organisms.³ Among them, seaweeds are a group of marine plants that are attached to the bottom in relatively shallow coastal waters. They are not as complex as that of the flowering plants, as they lack roots, flowers, seeds and true leaves. Yet within these structural limitations, seaweeds show diversity in shape, size, colour and structural complexity. More than 1, 50, 000 seaweed species are found in the marine environs of the globe though only a few of them have been identified and used for human purpose.

Recently, macro-algal metabolites are attracting the enormous attention, as they are known for their pharmacological properties. In addition, seaweeds are found to have different biological activities including antibacterial, antioxidant, antitumor, antiviral, antiprotozoal and anti-inflammatory characteristics.⁴ Free radicals have been reported to play an important role in affecting human health by causing many diseases such as, cancer, diabetes, heart diseases and hypertension. In the past decade, antioxidants have shown their relevance in the prevention of various diseases, in which free radicals have been, implicated.⁵ The potential protec-

tive effects of seaweeds against oxidative stress in target tissues and lipid oxidation in foods were reported earlier.⁶ Recently, some active antioxidant compounds from marine algae were reported *viz*; phylophoeophyllin in *Eisenia bicyclis*, phlorotannins in *Sargassum kjellmanianum* and fucoxanthin in *Hijikia fusiformis*. The sulphated polysaccharides of *Sargassum* act as a potent free radical scavenger as well as anticancer agent.⁷

Sargassum wightii Greville ex J. Agardh, 1848 and *Stoechospermum marginatum* (C. Agardh) Kützing 1843 are belonging to the class Phaeophyceae, and they are rich in phlorotannin and alginic acids, respectively.^{8,9} These seaweed species are widely distributed along the southeast coast of Tamil Nadu (India) and it is reported to be used as a source of sodium alginate, fertilizer, animal food, food ingredient and fertilizer. This alga is widely distributed in tropical and subtropical areas of central and western pacific and in Indian Ocean.

In view of this view, the present study was focussed on the antibacterial, antidiabetic, antioxidant and chemical characterization of methanolic crude extracts of brown seaweeds, *Sargassum wightii* and *Stoechospermum marginatum*.

MATERIALS AND METHODS

Chemicals

All the chemicals and organic solvents used in the study were maximum purity of analytical grade and purchased from Himedia, Mumbai, India.

Preparation of seaweed extracts

The samples of fresh brown marine algae, *Sargassum wightii* (Figure 1) and *Stoechospermum marginatum* (Figure 2) were collected from Mandapam, Ramanathapuram District, Tamil Nadu, Southeast coast of India (9°22' N; 78° 52' E). The collected seaweed samples were immediately washed with marine water. Then, the materials were brought to the laboratory and washed with tap water followed by sterile distilled water and the debris and associated biota were removed. The morphological characteristics of the collected algae have been ascertained based on standard keys (Dinabandhu, 2010).¹⁰ The reference specimens have been kept in the Department of Biotechnology, Periyar University, Salem, Tamil Nadu (India). The washed seaweed materials were shade-dried under shadow condition for 3 weeks. Dried and powdered seaweed materials of *S. wightii* and *S. marginatum* (100 g) were soaked into 300 ml of 100 % methanol and closed air tight and kept undisturbed for 10 days. Then the solvent could filter from seaweed debris through Whatman No.1 filter paper and the filtrates were concentrated by cold percolation method. Then the concentrated seaweed extracts were stored in 4°C for further characterization and applications.

Phytochemical screening

The freshly prepared methanol extracts of *S. marginatum* (SMME) and *S. wightii* (SWME) were subjected for preliminary phytochemical screening (saponin, phenolic compounds, sugar, tannins, alkaloids, steroids, flavonoid, glycoside, oils) according to the standard procedure.¹¹

Antibacterial assay

The antibacterial activities of the SMME and SWME were performed by the agar well diffusion method.¹² A volume of 15 ml of Muller Hinton agar plates were inoculated with 0.1 ml of overnight culture of the indicator strains. Wells of 7 mm in diameter were made in the agar and filled with different concentrations. The plates were incubated at 37°C for 24 h. Then, they were examined for the bacterial lawn and the diameters of the inhibition zones were measured.

Antidiabetic activity

α -amylase inhibitory activity

The α -amylase inhibitory activity of seaweed extracts was evaluated according to the standard procedure.¹³ Two hundred micro liter of starch (0.4 mg/ml) and 100 μ L of seaweed extracts (1 mg/ml) at varied concentration (6.25-100 μ g/ml) were dissolved in phosphate buffer (20 mM. NaH_2PO_4 and 6.7 mM. NaCl, pH 6.9). Consequently, 50 μ L of α -amylase solution (1 U/ml in phosphate buffer) was added to the sample mixture, after which phosphate buffer was added to obtain a final volume of 500 μ L. Then, the reaction mixture was incubated at room temperature for 3 min to allow the enzymatic reaction to occur. Finally, the reaction was terminated by adding 1 ml of 0.1% hydrochloric acid. Subsequently, iodine reagent (Wako amylase kit) was added to the mixture. The decrease in starch concentration due to the activity of α -amylase was measured at 660 nm using a multi-well ELISA plate reader (Tokyo, Japan).

α -glucosidase inhibitory activity

The α -glucosidase inhibitory activity of seaweed extracts was performed according to the standard method with slight modifications.¹⁴ In brief, 50 μ L of seaweed extracts (1 mg/ml) at different concentration (6.25-100 μ g/ml) and 50 μ L of 0.1 M phosphate buffer (pH 6.8) containing α -glucosidase solution (0.1 μ L/ml) were incubated at 37°C for 10 min. After, 50 μ L of 2.5 mM p-nitrophenyl- α -D-glucopyranoside (PNPG) solution was added in the same buffer and incubated for 20 min. Then, the reaction was terminated by adding 100 μ L of 0.2 M Na_2CO_3 and absorbance was recorded at 405 nm.

Antioxidant activity

DPPH free radical scavenging assay

The effect of extracts preparation on DPPH radical was assayed using the standard method.⁵ A methanolic solution of 0.5 ml of DPPH (0.4 mM) was added to different concentration of standard and extracts (6.25-100 μ g/ml), and allowed to react at room temperature for 30 min. The absorbance was measured at 518 nm in UV-visible Spectrophotometer (Cyber Lab UV-100, Millbury, MA). Methanol served as blank and DPPH in methanol without the sample served as the control.

Ferric reducing antioxidant power assay (FRAP)

The FRAP reagent contained 5 ml of TPTZ (2, 4, 6-tripyridyl- s- triazine) solution (10 mmol/L) in 40 mmol/L HCL with 5 ml of FeCl_3 (20 mmol/L) and 50 ml of acetate buffer, (0.3 mol/L, pH 3.6). The reagent was prepared freshly and warmed at 37°C. Aliquots of standard and extracts at different concentration (6.25-100 μ g/ml) were mixed with 3 ml FRAP reagent and the absorbance of reaction mixture read at 593 nm after the incubation of 10 min.⁶

Hydrogen peroxide scavenging assay

The Hydrogen peroxide scavenging activities of the extracts were determined by the method of Saha *et al.*⁵ Different concentration of standard and extracts (6.25-100 μ g/ml) were dissolved in 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and mixed with 600 μ L of 40 mM solution of hydrogen peroxide. The absorbance was recorded at 230 nm. For each concentration, a separate blank sample was used for background subtraction.

Inhibitory activity calculation and statistical analysis All the experiment was performed in triplicate. The *in vitro* antidiabetic and antioxidant activities of the seaweed extracts/standard were calculated using the following formula.

$$\text{Inhibition(\%)} = \frac{(A_c - A_s)}{A_c} \times 100$$

Where, A_c is absorbance of the control, A_s is absorbance of the sample.

High Performance Liquid Chromatography (HPLC)

The HPLC analysis of SWME and SMME was performed. About 1 mg of concentrated sample was dissolved in 1 ml of methanol and 20 μ L was injected to determine the Phyto-constituents. Methanol: water (50:50) was used as mobile phase. The experiment was performed in Shimadzu LC solution 20 AD (Japan, SPD 20 A), an instrument equipped with a UV detector to determine the peak purity. LCGC C18 column was used for isocratic resolution using the mobile phase at a flow rate of 1.0 ml/min. Using the detector shimadzu LC solution No. 20 AD, the developed plate was dried with a plate drier and subjected to UV analysis. All tracks in the plate were scanned at user defined wave length (254 nm) and individual RT values of each peak were obtained.¹⁵

Fourier Transformer Infrared Spectrophotometer (FT-IR)

The FTIR analysis of SWME and SMME were performed using FTIR spectrometer coupled with TGS (Tri-glycine sulphate) detector (Bruker, D8, Germany model). In brief, 1 mg of each dried sample was mixed with 100 mg of potassium bromide (KBr) and then compressed to prepare salt-disc (3 mm dia). These discs were recorded in the mid-IR region 4000-400 cm^{-1} at room resolution 4 cm^{-1} .¹⁶

Gas Chromatography-Mass Spectrometer

GC-MS analysis of SWME was performed by injecting 1 μ L of sample on a DB-1 capillary column of GC-MS model (GC-MS) QP 2010 with a QP-5000 mass spectrometer (both from Shimadzu) and Helium was used as mobile phase with a constant flow-rate of 1.0 ml/min. The qualitative and quantitative analyses of the sample were carried out using a CP 3800 Saturn 2200 GC-MS system. The GC-MS operating parameter as fol-

lows, program temperature: initial temperature was 50°C for 3 min, then gradually increased to 250°C at the rate of 10°C/min, finally maintained for 3 min; Ion temperature: 200°C and scan range: 20-500 AMU (Atomic Mass Unit). The identification of components was based on comparison of their mass spectra with those of Wiley library.¹⁷

RESULTS AND DISCUSSION

Phytochemical analyses

Preliminary phytochemical analyses of SWME revealed the presence of major Phyto-constituents like carbohydrates, glycosides, saponins, flavonoids, fixed oil, fat, phenol compounds, tannins and steroids. The Phyto-constituents such as, phenols and flavonoids were found to enhance the scavenging of free radicals, as reported by earlier researchers.¹⁸ And the SMME has resulted the phytochemicals carbohydrates, glycosides, alkaloids, amino acids, fixed oil, fat, phenol compounds, tannins and steroids (Table 1).

Antibacterial activity

The results of the antibacterial activity of SWME and SMME are given in the Table 2. The zones of inhibition were measured after 24 h of incubation and the findings are shown in Figure 3 and Table 2. Rodriguez *et al.*¹⁹ have reported that, seaweed is an excellent source for compounds such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids that show different biological activities. In the present study, SMME showed maximum inhibition and SWME showed only moderate activity against *E. coli* and *S. aureus*. These results are in agreement with the previous findings of Abhary and Al-Hazmi.²⁰ Recently, an highest zone of inhibition (18.1 mm) was observed in methanol extract of green alga, *Pithophora oedogonia* against Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*.²¹ The methanolic extracts of six marine algae that belonged to Rhodophyceae (*Corallina officinalis*), Phaeophyceae (*Cystoseria barbata*, *Dictyota dichotoma*, *Halopteris filicina*, *Cladostephus spongiosus*, *F. verticillatus*) and Chlorophyceae (*Vulva rigida*) showed good antibacterial activity against pathogenic microbes i.e., three Gram positive bacteria *viz*; *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis* and three *g* negative bacteria *viz*; *Escherichia coli*, *E. aerogenes*, *E. coli* through *in vitro* antibacterial assay were reported.²² The presence of phenolic compounds in seaweeds may affect growth and metabolism of bacteria.²³ They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration.



Figure 1: *Sargassum wightii* Greville ex J.Agardh.

Antidiabetic activity

α -amylase inhibitory activity

The most common therapeutic approach to treat diabetes is reduction of postprandial hyperglycemia. This can be achieved by inhibition of enzymes involved in the release of glucose from foods and this approach is used in the management of type 2-diabetes, with the main target being α -amylase and α -glucosidase.²⁴ Alpha-amylase is an enzyme responsible for the breakdown of complex carbohydrate like starch to more simple sugars like glucose. Thus, the inhibition of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption. Natural α -amylase inhibitors offer an attractive therapeutic approach in the treatment of postprandial hyperglycemia, by ultimately decreasing glucose release from starch. The alpha amylase inhibitory findings of both SWME and SMME are presented in Figure 4. The inhibitory capacities of seaweed extracts against α -amylase was found to be minimum (IC₅₀ SWME: 58.36 μ g/mL) and moderate IC₅₀ values (SMME: 195.1 μ g/mL). The positive control, acarbose has revealed the maximum activity with the IC₅₀ value of 4.023 μ g/mL.

α -glucosidase inhibitory activity

The alpha glucosidase inhibitory properties of both SWME and SMME are presented in Figure 4. Only moderate inhibitory capacities of seaweed extracts against α -glucosidase was recorded (IC₅₀ values (μ g/mL): 109.5 and 171.1, respectively). The positive control acarbose has revealed the maximum activity with the IC₅₀ value of 22.95 μ g/mL.

In vitro antioxidant activity of seaweed crude extracts

DPPH radical scavenging assay

The DPPH radical scavenging capacity of SWME and SMME were evaluated at different concentrations (20-100 μ g/ml) of the extracts and the results are illustrated in Figure 5. The SWME exhibited a strong DPPH activity (87.86% \pm 0.56), followed by SMME (84.71% \pm 0.17 %). The positive control ascorbic acid exhibited very high range of DPPH scavenging activity when compared to the crude extracts (94.38% \pm 1.21%). The IC₅₀ values of the DPPH scavenging property of the SWME and SMME and ascorbic acid were: 64.51, 72.81 and 60.81 μ g/ml respectively. The earlier report of antioxidant activity of three edible species of *Ulva* (*U.*



Figure 2: *Stoechospermum marginatum* (C.Agardh) Kützing 1843.

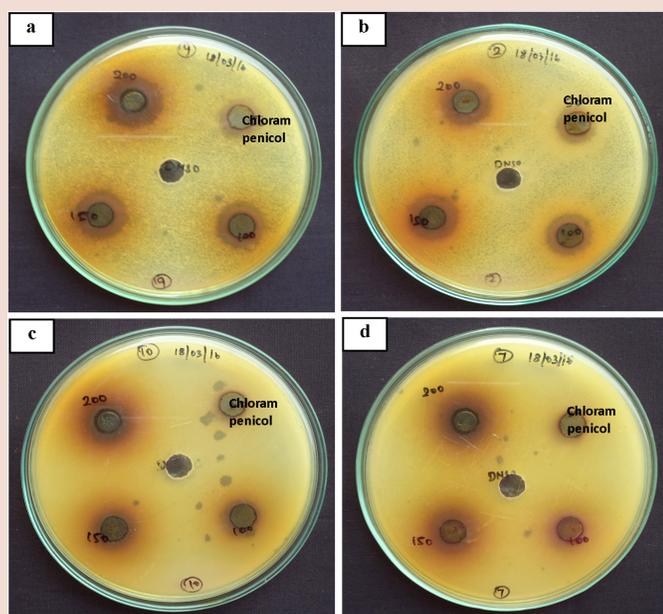


Figure 3: Antibacterial activity of SWME and SMME against (a, b) *E. coli* & (c, d) *S. aureus*.

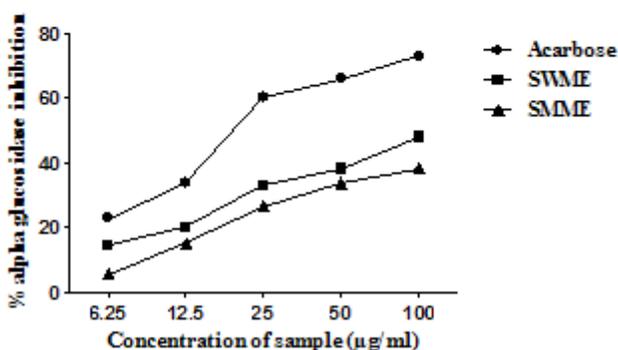
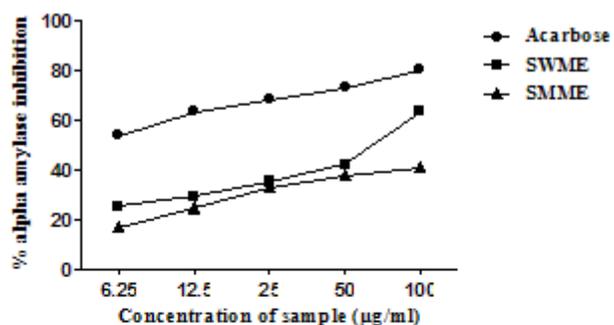


Figure 4: Antidiabetic activity of SWME and SMME against α -amylase and α -glucosidase.

compressa, *U. linza* and *U. tubulosa*) have exhibited high antioxidant activity in linoleic acid system and the best DPPH radical scavenging was observed in methanolic extract of *U. compressa* ($IC_{50} = 1.89 \text{ mg ml}^{-1}$) and the present study revealed the same.²⁵ The presence of antioxidant substances in seaweeds is found to be an endogenous defence mechanism as

a protection against oxidative stress due to extreme level of environmental conditions.²⁶

FRAP radical scavenging assay

The ferric ion reducing capacity of the SWME and SMME and control are illustrated in Figure 5. The SWME ($85.95\% \pm 1.02$) exhibited a strong FRAP activity, followed by the SMME ($81.03\% \pm 0.15$). The positive control ascorbic acid was found to have very high range of FRAP scavenging activity when compared to the crude extracts ($94.79\% \pm 0.74$). A report of an earlier study suggests that the antioxidant activity of the brown algal species is significantly correlated with the quantity of phenolic content present in the seaweed.²²

H_2O_2 radical scavenging assay

The antioxidant property of methanol extracts of SWME and SMME on hydrogen peroxide is shown Figure 5. The extracts of SWME and SMME were capable of scavenging hydrogen peroxide in an amount dependent manner. $100\mu\text{g/ml}$ of methanol extracts of SWME exhibited $95.7 \pm 0.59\%$ scavenging activity on hydrogen peroxide followed by the methanol extract of SMME $90.11 \pm 0.29\%$. On the other hand, using the same amounts, ascorbic acid exhibited $98.93 \pm 0.47\%$ of hydrogen peroxide scavenging activity. The results of antioxidant activities of various assays (DPPH, FRAP and H_2O_2) showed that, *S. wightii* has higher radical scavenging property when compared to the *S. marginatum*. Presently, the SWME and SMME showed excellent antioxidant activity as reported earlier.²⁷ The antioxidant activity of brown seaweed extracts correlated with their polyphenol content which might be the cause of recorded inhibitory effects.²⁸

Purity analysis by HPLC

The extracts were initially tested for their purity using reverse phase HPLC analysis. C18 column was used as stationary phase and methanol: water (50:50) ratio was used as mobile phase (Shimadzu LC2010A, Japan). 1 mg of extract was weighed and dissolved in 1 ml DMSO and further it was diluted in methanol to get the concentration of 1mg/ml stock. Initially, $20\mu\text{l}$ of samples were injected the HPLC C18 column and the extracts were detected by UV detector. Percentage of purity was calculated after subtraction of contamination peaks in the chromatogram (Figure 6).

FTIR spectral analysis of SWME and SMME

The FTIR was used to identify the functional group of the active components based on peak value of the infrared radiation. When the methanol extracts of SWME and SMME were passed into the FTIR, the functional groups of the components got separated based on peak ratio. The FTIR spectroscopic studies have revealed the presence of various functional groups in SWME and SMME (Figure 7). The FTIR spectrum of SWME showed bands at 3157.79 , 2929.41 , 2351.71 , 1638.15 , 1418.83 , 1112.03 and 666.02 cm^{-1} . The band at 3157.79 and 2929.41 cm^{-1} could be assigned to the $-\text{CH}_2-$ stretching vibration of alkanes groups. The band at 2351.71 cm^{-1} corresponds to the P-H phosphine stretching vibration of miscellaneous groups. The bands at 1638.15 cm^{-1} could be attributed to the C=O stretching vibration of amides. The bands at 1418.82 , 1254.05 and 1112.03 cm^{-1} signals the S=O sulphate ester and P-H bending vibration of miscellaneous groups, respectively. The band at 666.02 cm^{-1} corresponds to the N-H wag amines stretching vibration of amines groups. FTIR spectrum of SMME showed the presence of bands at 3351.84 , 2929.08 , 2862.09 , 2329.93 , 1728.60 , 1456.61 , 1376.06 , 1254.05 , 1181.20 , 1051.72 and 898.60 cm^{-1} . The bands at 3351.84 , 2929.08 and 2862.09 cm^{-1} could be assigned to the CH stretching vibration of alkanes groups. Similarly, the band at 2329.93 cm^{-1} signals the Phosphine sharp stretching vibration of miscellaneous groups. The band at 1728.60 cm^{-1} also represents the C=O stretching vibration of aldehyde. The bands at 1456.61

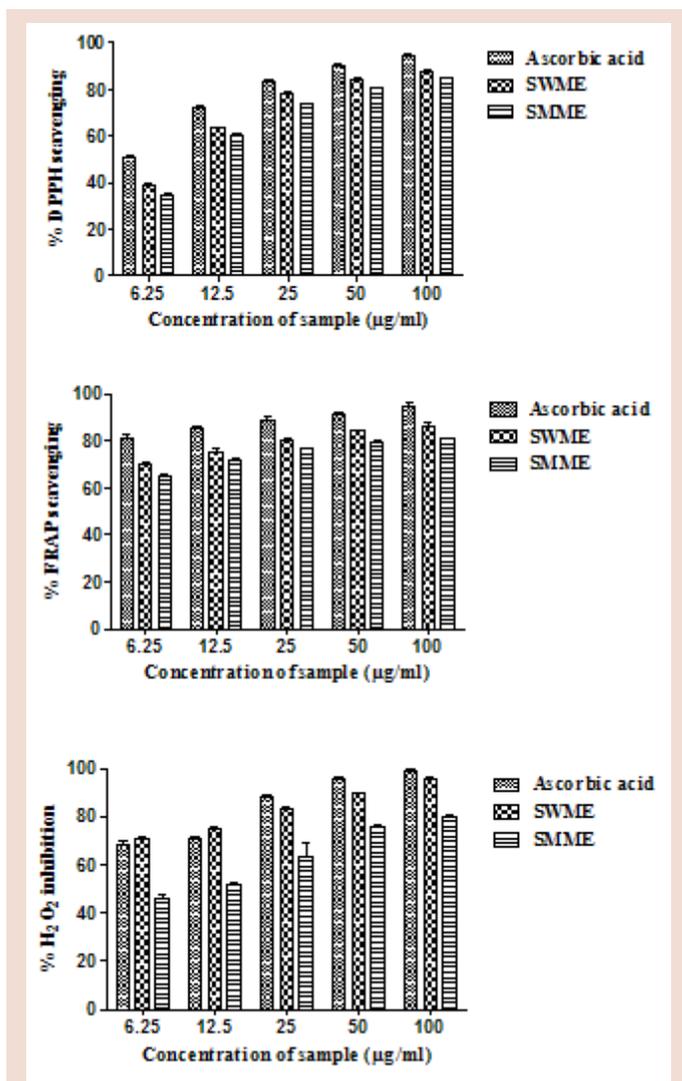


Figure 5: Antioxidant activities of SWME and SMME.

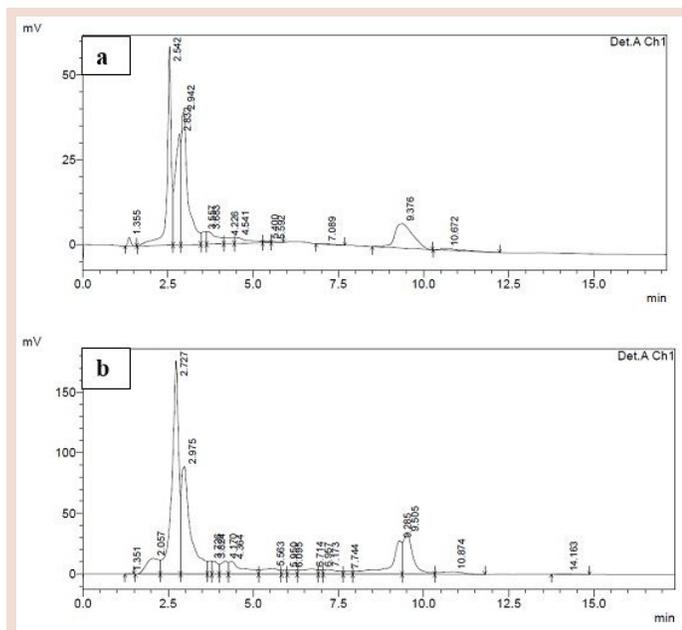


Figure 6: HPLC analyses of a. SWME and b. SMME.

Table 1: Phytochemical analysis of SWME and SMME

Phytochemicals	SWME	SMME
Carbohydrates & glycosides	+	+
Proteins & amino acids	+	+
Saponins	+	-
Fixed oil & fat	+	+
Tannin	+	+
Flavonoids	+	-
Alkaloids	+	+
Steroids	+	+
Phenol compounds	+	+

Table 2: Antibacterial activities of SWME and SMME

Bacteria	Sample	Concentration (µg/mL)			
		Chloramphenicol	100	150	200
<i>E. coli</i>	SWME	14.1±0.23	17.3±0.36	19.1±0.76	22.0±0.36
	SMME	13.0±0.56	15.2±0.33	17.0±0.33	18.3±0.84
<i>S. aureus</i>	SWME	-	11.0±0.23	12.5±0.57	13.4±0.57
	SMME	-	-	10.0±1.08	12.3±0.22

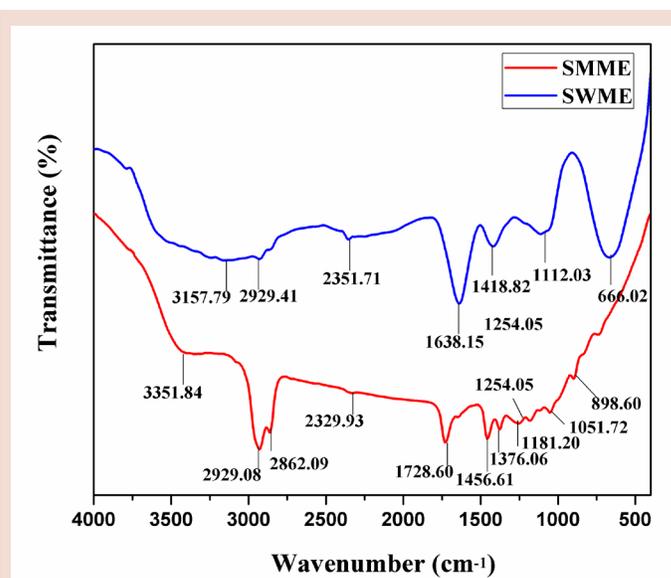


Figure 7: FTIR analyses of a. SWME and b. SMME.

and 1376.06 cm^{-1} consigned to the CH_2 and CH_3 stretching vibration of alkanes groups. The band at 1254.05 cm^{-1} also represents the C-H wag ($-\text{CH}_2\text{X}$) stretching vibration of Alkyl halides. The bands at 1181.20 and 1051.72 cm^{-1} were signalling to the C-N stretching vibration of amines. The band at 898.60 cm^{-1} signals the S-OR esters of miscellaneous groups. In addition, the recorded, FTIR peaks indicate the presence of alcohols, alkynes, aromatics, carboxylic acids and alkyl halides. A similar finding was reported earlier which showed the functional groups from bioactive compounds extracted from methanolic extracts of *S. wightii* and *U. lactuca*.²⁹

Gas chromatography and mass spectrometer (GC-MS)

The GCMS analysis of SWME indicated the presence of 20 peaks (Figure 8). The major compounds of SWME were hexadecanoic acid, methyl

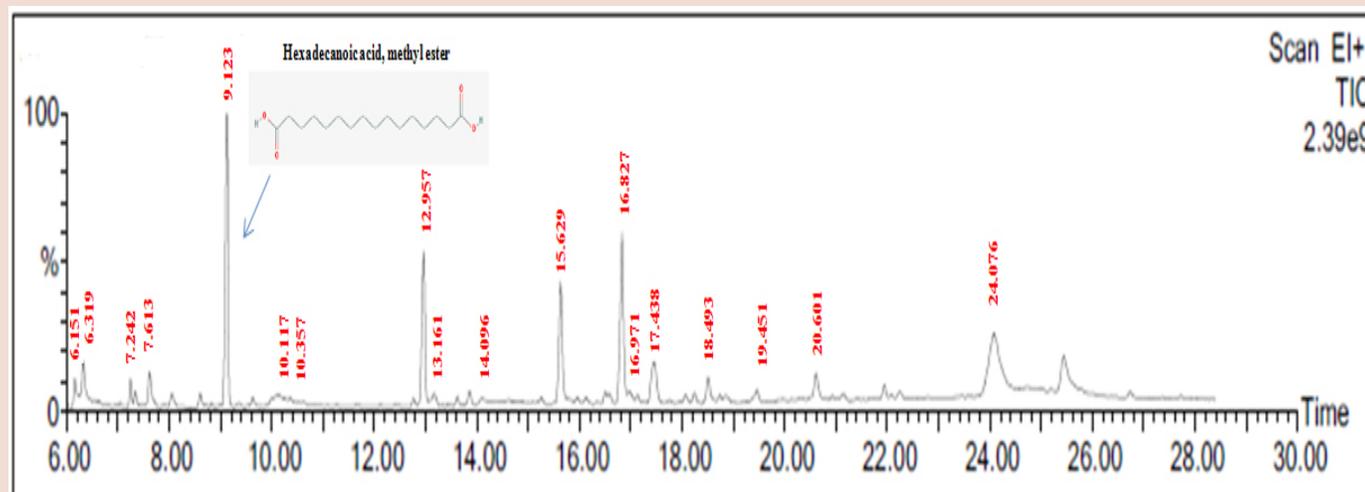


Figure 8: GC-MS analysis of SWME.

Table 3: Chemical compounds of SWME

S.No	R _T	Compound name	MW	MF	Peak area (%)	Biological activity
1	3.731	1,11-Hexadecadiyne	218.0	C ₁₆ H ₂₆	1.111	No activity found
2	5.660	Methyl tetradecanoate	242.0	C ₁₅ H ₃₀ O ₂	2.022	Antioxidant & antiCancer [29]
3	6.151	trans-Z-à-Bisabolene epoxide	220.0	C ₁₅ H ₂₄ O	1.565	Anti-inflammatory [33]
4	6.319	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	168.0	C ₁₁ H ₂₀ O	3.329	Hepatotoxicity & oxidative Stress [34]
5	7.242	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296.0	C ₂₀ H ₄₀ O	0.882	Antimicrobial & anti-inflammatory [35]
6	7.613	Aromadendrene oxide-(2)	220.0	C ₁₅ H ₂₄ O	1.894	Antioxidant [36]
7	9.123	Hexadecanoic acid, methyl ester	270.0	C ₁₇ H ₃₄ O ₂	13.359	Antioxidant & Nematicide [30]
8	10.117	n-Hexadecanoic acid	256.0	C ₁₆ H ₃₂ O ₂	2.456	Antibacterial & antifungal [31]
9	10.357	Guaia-9,11-diene	204.0	C ₁₅ H ₂₄	0.929	Antifungal & antitumor [37]
10	12.957	9-Octadecenoic acid (Z)-, methyl ester	296.0	C ₁₉ H ₃₆ O ₂	7.792	Anti-cancer [38]
11	13.161	Phytol	296.0	C ₂₀ H ₄₀ O	1.090	Antimicrobial & anticancer [30]
12	14.096	Hexadecenoic acid, Z-11-	254.0	C ₁₆ H ₃₀ O ₂	1.030	Antioxidant [30]
13	15.629	Isoaromadendrene epoxide	220.0	C ₁₅ H ₂₄ O	7.384	Antibacterial activity [39]
14	16.827	Isoaromadendrene epoxide	220.0	C ₁₅ H ₂₄ O	9.883	Antibacterial activity [39]
15	16.971	3-Hexen-1-ol, 2,5-dimethyl-, acetate, (Z)-	170.0	C ₁₀ H ₁₈ O ₂	0.892	No activity found
16	17.438	1-Heptatriacotanol	536.0	C ₃₇ H ₇₆ O	3.884	Anticancer [33]
17	18.493	10-Methyl-8-tetradecen-1-ol acetate	268.0	C ₁₇ H ₃₂ O ₂	1.915	Antidiarrhoeal [34]
18	19.451	Isoaromadendrene epoxide	220.0	C ₁₅ H ₂₄ O	1.278	Antibacterial activity[39]
19	20.601	Retinal	284.0	C ₂₀ H ₂₈ O	2.212	No activity found
20	24.076	1-Heptatriacotanol	536.0	C ₃₇ H ₇₆ O	11.885	Anticancer [33]

ester (13.35%), 1-heptatriacotanol (11.885), isoaromadendrene epoxide (16.827), 9-octadecenoic acid (Z)-, methyl ester (12.957). The remaining compounds are minor components, the name of the compounds; retention times, molecular weight, molecular formula, and their percentages are listed in Table 3. In the present study, SWME has shown the major peak for hexadecanoic acid, which has been previously, reported for antibacterial, antioxidant, antifungal, nematicide, anti-inflammatory, hypocholesterolaemia, pesticide, anti-androgenic flavor, hemolytic, 5- α reductase inhibitor and mosquito larvicide.^{30,31,32} Hence, the recorded

activities of antibacterial, antidiabetic and antioxidant activity of the SWME might be due the presence of the phyco-chemical, hexadecanoic acid.

CONCLUSION

Presently recorded maximum antioxidant, antibacterial and antidiabetic activity of the *S. wightii*-extracts might be due to its Phyto-constituents, as revealed by GC-MS analysis. The isolation and purification of the

compounds from SWME may be useful for the future pharmacological studies.

CONFLICT OF INTEREST

None.

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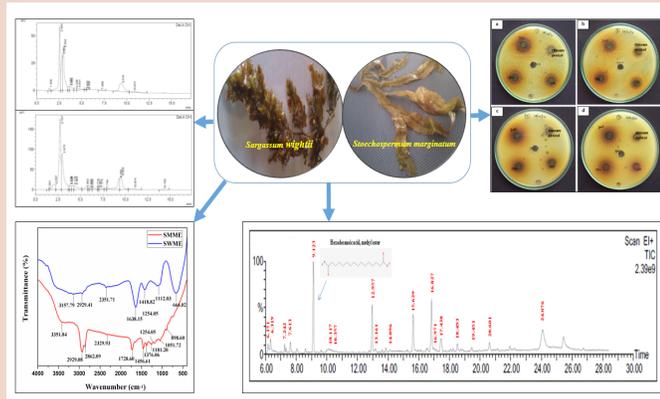
ABBREVIATIONS USED

FT-IR: Fourier Transformer Infrared Spectrophotometer; GC-MS: Gas Chromatography and Mass Spectrometer; HPLC: High Performance Liquid Chromatography; SMME: methanolic extract of *Stoechospermum marginatum*; SWME: methanolic extract of *Sargassum wightii*; FRAP: Ferric reducing antioxidant power assay; TGS: Tri-glycine sulphate.

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PICTORIAL ABSTRACT



SUMMARY

- In our study we observed the methanolic extract of *S. wightii* have showed maximum activity than the *S. marginatum*.
- DPPH scavenging of SWME exhibited a strong activity (87.86% ± 0.56) followed by SMME (84.71% ± 0.17 %).
- FRAP assay of SWME (85.95% ± 1.02) exhibited a strong activity, followed by the SMME (81.03% ± 0.15).
- *In vitro* alpha amylase and alpha glucosidase inhibitory of SWME showed maximum activity than the SMME.
- GC-MS analysis of SWME has shown the major peak for hexadecanoic acid.

ABOUT AUTHOR



Paramasivam Deepak: Is a full time Ph.D. research scholar at Department of Biotechnology, Periyar University, Salem-636011, Tamil Nadu, India. He obtained his M.Sc and M.Phil degrees in the same University. He has secured first rank in his M.Sc – Biotechnology (2014) from Periyar University. Currently he is working as University Research Fellow (URF) in the same University. His focused area of research interest is isolation of marine bioactive compounds as an effective alternative medicine for Diabetes mellitus.



Muthu Paulraj Diviya Josebin: Is a full time Ph.D. research scholar at Department of Microbial Biotechnology, Bharathiar University, Coimbatore-641046, Tamil Nadu, India. She has completed her M.Sc and M.Phil (Biotechnology) degrees in Periyar University, Salem-636011, Tamil Nadu, India. Her focused area of research interest is Bioremediation.



Rajasekaran Kasthuridevi: Did her M.Sc (Biotechnology) at the Department of Biotechnology, Periyar University, Salem-636011, Tamil Nadu, India. She did her M.Sc-Project work on antioxidant activities of marine brown seaweeds.



Dr. Rajamani Sowmiya: Did her Ph.D. in Biotechnology at the Department of Biotechnology, Periyar University, Salem-636011, Tamil Nadu, India. She obtained her M.Sc and M.Phil degrees from the same University. She did her Ph.D. research work on antiplasmodial activity of marine seaweed compounds. She is currently working as a senior research scientist at Greens med labs, Thoraipakkam, Chennai-600 097, Tamil Nadu, India.



Govindasamy Balasubramani: Is a full time Ph.D. research scholar at Department of Biotechnology, Periyar University, Salem-636011, Tamil Nadu, India. He has been obtained his M.Sc and M.Phil degrees from the same University. His Ph.D. research work deals with the isolation and identification of Quorum sensing bacteria from gut of infected fish.



Dilipkumar Aiswarya: Is a full time Ph.D. research scholar at Department of Biotechnology, Periyar University, Salem-636011, Tamil Nadu, India. She has obtained her M.Sc degree from the same University. She had secured first rank in her M.Sc - Biotechnology from Periyar University. She has been awarded DST-Inspire Fellowship (JRF) for Ph.D research. Her focused area of research interest is entomopathogenic nematodes as biocontrol agents.



Prof. Dr. Pachaiappan Perumal: Is a Professor and Head of the Department of Biotechnology, Periyar University, Salem-636011, Tamil Nadu, India. He has passed the UGC-National Educational Test (1985) and he earned his doctorate from CAS in Marine Biology, Annamalai University. He has been specializing in the areas of Marine Biotechnology, Molecular taxonomy/ oncology and Bio-nanotechnology. He has over 150 publications to his credit and has handled/completed over dozen research projects.